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Isolation of *hMRE11B*: failure to complement yeast *mre11* defects due to species-specific protein interactions

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Abstract

The *Saccharomyces cerevisiae MRE11* gene plays an important role in meiotic recombination, mitotic DNA repair and telomere maintenance. We present the isolation of *hMRE11B* cDNA from a human HeLa cell cDNA library as an *MRE11* homolog. Compared to the previously identified *hMRE11*, *hMRE11B* contains an additional 84 bp sequence that results in a 28 amino-acid insertion close to the C-terminus. The expression pattern of *hMRE11B* in different tissues shows the presence of two mRNA species of approx. 2.6 and 7.5 kb. Overexpression of *hMRE11B* does not complement the alkylation sensitivity of the *mre11* null and temperature-sensitive mutant strains. In this study, we examine factors that may explain this lack of complementation. First, both Northern and Western analyses rule out the lack of *hMRE11B* transcription and/or translation in yeast. Second, we demonstrate that hMre11B, like the yeast Mre11 protein, dimerizes in vivo in a yeast two-hybrid system. This dimerization requires the C-terminal one-third of hMre11B protein, which includes the 28 amino acids absent in hMre11. However, hMre11B does not interact with Mre11, Rad50 and Xrs2. Hence, the lack of protein–protein interaction between hMre11B and the yeast Mre11, Rad50, and Xrs2 may explain the inability of *hMRE11B* to complement the yeast *mre11* mutants. We rule out the hypothesis that the lack of interaction and, in turn of complementation, is due to the absence of sequence homology at the C-terminal domain of hMre11B compared to the yeast Mre11. Instead, we propose that the C-terminus of hMre11B participates in protein–protein interaction and functions in a species-specific manner. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Mre11; Protein–protein interaction; Recombinational repair

1. Introduction

Genetic information is maintained by DNA repair pathways and the fidelity of these processes is crucial for the maintenance of the genetic integrity of living organisms. In yeast, DNA repair genes are classified into three epistasis groups: *RAD3* nucleotide excision repair, *RAD6* post-replication repair and *RAD52* recombination repair (Friedberg et al., 1995). Genes in the *RAD52* group are responsible for repair of DNA strand breaks and other damage that causes replication block during vegetative growth, and most of them function during different stages of meiosis (Petrini et al., 1997). Within the *RAD52* epistasis group, *RAD50*, *MRE11* and *XRS2* genes are believed to function at an early stage of meiotic recombination where double-strand breaks are formed and processed into proper intermediates for the subsequent steps of recombination to proceed (Johzuka and Ogawa, 1995). In a recent study, epistasis analysis showed that during mitotic growth, the Mre11/Rad50 complex seems to function to prepare DNA ends for telomerase to replicate (Nugent et al., 1998). The *rad50*, *mre11* and *xrs2* mutants display similar phenotypes in terms of meiotic recombination frequency and spore formation, and sensitivity to simple DNA alkylating agents and ionizing radiation (Alani et al., 1990; Ivanov et al., 1992; Johzuka and Ogawa, 1995). These mutants also display a hyper-recombination phenotype in some mitotic recombination assays (Malone et al., 1990; Ajimura et al., 1993). Yeast Mre11 has been shown to interact with Rad50 and Xrs2 in a yeast two-hybrid assay (Johzuka and Ogawa, 1995).

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Abbreviations: DMSO, dimethyl sulfoxide; DSB, double-strand breaks; MRE11-Ch, chimeric *MRE11*; MTN, multiple tissue northern; PCR, polymerase chain reaction; PolIk, Klenow (large) fragment of *Escherichia coli* DNA polymerase I; SD, synthetic dextrose; ts, temperature sensitive; yMre11, yeast Mre11.

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Hence, these three proteins probably form a complex in order to function during meiosis as well as mitotic DNA repair.

In all eukaryotic organisms examined to date, DNA repair proteins, especially those involved in nucleotide excision repair (Hoeijmakers, 1993; Aboussekhra et al., 1995) and recombinational repair (Petrini et al., 1997) are highly conserved both structurally and functionally. However, the functions of the genes encoding these proteins appear to be species-specific. For example, although the average protein sequence similarity between human Rad51, Rad52, Rad54 and their yeast counterparts exceeds 60%, they are unable to fully complement the corresponding yeast null mutants (Shinohara et al., 1993; Kanaar et al., 1996; Donovan et al., 1994). It is not known, however, if the human proteins constitute a higher order complex that differs from the lower eukaryotes or the functional or interacting domains of these proteins are less conserved. Analysis of protein–protein interaction between hRad51 and hRad52 revealed that the region of hRad52 protein responsible for interacting with hRad51 shows no homology with the yeast Rad52 protein, indicating that this interaction is species-specific (Shen et al., 1996).

A yeast *ngs1* mutant was previously isolated by its enhanced sensitivity to simple DNA alkylating agents such as methyl methanesulfonate (MMS) (Nisson and Lawrence, 1986). We cloned the yeast *NGS1* gene by functional complementation of the *ngs1* mutant and found that *NGS1* is identical to *MRE11* (Chamankhah and Xiao, 1998). Using the primary sequence of *MRE11*, we then cloned the human homolog, which we call *hMRE11B* to distinguish our clone from the previously reported *hMRE11* cDNA (Petrini et al., 1995). Our *hMRE11B* does not complement the DNA alkylation repair and recombination defects of the yeast *mre11Δ* and *mre11-ts* strains. Several hypotheses were examined to determine the reasons for this lack of conservation of function. Our results support the notion that species-specific protein interaction determines the functional specificity of *MRE11* and that the participation of the C-terminus of Mre11 protein plays an important role in this regard.

2. Materials and methods

2.1. Strains and media

Saccharomyces cerevisiae haploid strain DBY747 was originally obtained from Dr D. Botstein (Stanford University, USA). The *mre11Δ* strain was constructed using the one-step gene replacement method (Rothstein, 1991). Briefly, DBY747 wild-type cells were transformed with the *mre11* disruption cassette, which was linearized prior to transformation by treating plasmid pMCY77 with *Hin*dIII–*Nde*I. pMCY77 was made by replacing the 2.3 kb *Afl*II–*Nru*I fragment of the yeast *MRE11* gene with a 2.9 kb *LEU2* gene. Clones that displayed the cosegregation of the URA^+ MMS^S phenotype were subjected to Southern analysis to confirm the disruption of the *MRE11* gene. Strain E8-1 carrying a temperature-sensitive allele of *MRE11 (mre11-ts)* was received from Dr H. Ogawa (Osaka University, Japan). Yeast cells were grown in either complete YPD medium (1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose, 2% Bacto-agar) or synthetic dextrose (SD) medium (0.67% Bacto-yeast nitrogen base without amino acids, 2% glucose, 2% Bacto-agar). When necessary, nutritional supplements were added to SD medium as recommended (Kaiser et al., 1994).

2.2. Screening a cDNA library

Detailed cloning of the *hMRE11B* cDNA is described in the Results section. In order to search for the full-length *hMRE11B*, a HeLa cell cDNA library made in pSPORT1 (Gibco BRL) was used to transform *Escherichia coli* DH5α cells by electroporation (Sambrook et al., 1989). Approximately 5×10^5 colonies were screened by colony hybridization using the 0.5 kb 5′ end of the HGS360.301 insert as a probe. A total of 16 putative *hMRE11B* clones (HeLa 1–16) were obtained, among which the amino-acid sequence deduced from one of these clones, HeLa16, demonstrated extended homology to the N-terminal yMre11 and contained the full-length *hMRE11B* cDNA.

2.3. Plasmid construction and DNA sequencing

The *hMRE11B* cDNA sequence was determined by the dideoxy-chain termination method (Sanger et al., 1977) using the T7 Sequencing Kit (Pharmacia LKB) as instructed. In order to examine the expression of *hMRE11B* cDNA in yeast, plasmid YEp-hMRE11B was constructed by inserting the 2.6 kb *Kpn*I–*Sna*BI fragment of the entire HeLa16 cDNA insert into the *Kpn*I–*Sma*I sites of YEp127. YEp127 was made by replacing the *GAL1* promoter from pYES2.0 (Invitrogen) with a constitutive *ADH1* promoter. In pGBT-yMRE11 and pGAD-yMRE11, the 2.3 kb *yMRE11* ORF was cloned in frame in the multiple cloning sites of pGBT9 and pGAD424, respectively. pGAD-yRAD50 was made by cloning the 4.0 kb *Eco*47III–*Sal*I fragment of pNKY74 (received from Dr N. Kleckner, Harvard University) into the *Sma*I–*Xho*I site of pACTII-Nc. To make pGAD-yXrs2, the 2.7 kb *Bam*HI fragment of pEI43 (received from Dr J. Haber, Brandeis University) was cloned into the *Bam*HI site of pGAD424. Plasmids pGBT-hMRE11B and pGAD-hMRE11B were made by cloning the 2.4 kb *Pst*I fragment of HeLa16 cDNA into the *Pst*I site of both pGBT9

and pGAD424 (Bartel and Fields, 1995), respectively. In order to make C-terminal, N-terminal, and internal deletions of *hMRE11B*, pGBT-hMRE11B was cleaved with *Sma*I–*Cla*I, *Bgl*II–*Spe*I or *Bgl*II, followed by treatment (if necessary) with PolIk and religation. The new clones were named pGBT-hM11Sm-Cla, pGBT-hM11Bg-Spe and pGBT-hM11Bg, respectively. The same procedure was applied to form pGAD-hM11Bg. The chimeric *5′-hMRE11-3′-yMRE11* clone, pGBTMRE-Ch1, was constructed in several steps. First, the C-terminus of *yMRE11* in pGAD-yMRE11 was PCR amplified using MRE11-Ch1 primer with the sequence 5′-GGCAGCTGTTTGATGTATTACAA-TCAG-3′ (*Pvu*II site underlined) and the *ADH1* terminator primer JN069 with the sequence 5′-TTG-ATTGGAGACTTGACC-3′. pGAD-hM11Bg was subjected to *Sma*I–*Sal*I double digestion followed by treatment with PolIk and self-ligation to remove the *Sal*I site while restoring the reading frame. Next, the 1.3 kb *Eco*RI–*Bgl*II fragment of this modified pGAD-hM11Bg containing the N-terminal hMRE11B coding region was isolated and cloned into the *Eco*RI–*Bam*HI sites of pGBT9$_{Pv}$ (a derivative of pGBT9 where a unique *Pvu*II site was destroyed by *Pvu*II cleavage and insertion of a *Hin*dIII linker with the sequence 5′-CCC-AAGCTTGGG-3′) to give pGBT-hM11-EBg. The 0.5 kb *Pvu*II–*Sal*I fragment of pGBT-hM11-EBg was then replaced by the 1.4 kb *Pvu*II–*Sal*I fragment of the PCR product of yeast *MRE11* from pGBT-yMRE11. The chimeric *5′-yMRE11-3′-hMRE11* clone, pGBTMRE-Ch2 was constructed as follows. First, pGBT9$_{Pv}$-hMRE11B was made by cloning the 2.4 kb *Pst*I fragment of HeLa16 cDNA into the *Pst*I site of pGBT9$_{Pv}$. Next, the N-terminus of *yMRE11* in pNGS1-4 was PCR amplified using MRE11-Ch2 primer with the sequence 5′-GGCAGCTGAATTGGATTGTGTA-CGAG-3′ (*Pvu*II site underlined) and the MRE11-Ch3 primer with the sequence 5′-CCGTCGACCT ATGGACTATCCTGATC-3′ (*Sal*I site and the *yMRE11* translation start codon underlined). Finally, the 0.45 kb *Pvu*II–*Sal*I fragment of this clone was replaced by the 0.45 k *Pvu*II–*Sal*I fragment of the PCR product. Both pGBTMRE-Ch1 and pGBTMRE-Ch2 structures were confirmed by sequencing the critical regions.

2.4. Measurement of sensitivity to MMS

Liquid killing experiments were carried out as follows. Yeast cells were transformed with YEp-hMRE11B or the chimeric *MRE11* clones using a DMSO transformation protocol (Hill et al., 1991). Yeast cells were inoculated in 5 ml SD-Ura selective media overnight, a 200–500 μl aliquot was transferred to 5 ml fresh YPD. Cells were cultured for several hours until the cell titer was approx. $2–5 \times 10^7$ cells/ml. MMS at the given concentration was added and samples were taken every 20 min, treated with fresh solutions of sodium thiosulfate (5% w/v) to neutralize MMS, and washed once more with sterile distilled water. Cells were resuspended in sterile water and serial dilutions were made and plated on YPD plates. Colonies were scored after 3–5 days of incubation of plates at either 30°C or 34°C (*mre11-ts* strain). Alternatively, yeast strains were grown to stationary phase in selective media, diluted and plated on YPD plates containing different concentrations of MMS. To assess the MMS sensitivity of the *mre11-ts* mutant strain (E8-1), 24°C was used as the permissive and 34°C as non-permissive temperature.

2.5. In vivo assay of protein interaction using yeast two-hybrid system

Yeast haploid strain Y190 (a gift from Dr D. Gietz, University of Manitoba, Canada) was used for all two-hybrid assays. A filter assay was employed to determine the β-galactosidase activity. Briefly, five to ten independent co-transformants with both Gal4 binding and activation domain fusion constructs were plated on SD-Trp-Leu selective media for 1–2 days. Cells were transferred to Whatman No. 1 filter paper, immersed in liquid nitrogen for 10 s to permeabilize cells, and placed on top of another filter which was presoaked in a mixture of 1.8 ml Z-buffer (Guarente, 1983), 5 μl β-mercaptoethanol and 45 μl of 20 mg ml X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) in *N,N*-dimethylformamide. Plates were sealed using parafilm and incubated at 30 C. Color development was monitored at 4, 8, 16 and 24 h after incubation. Clones that developed visible blue color by 4 h were scored as 3+, 8 h as 2+, 16 h as 1+ and 24 hr as +/−. Clones remaining white after 24 h were considered negative. Y190 cells transformed with pGBT9 and pGAD424 vectors were used as the negative control.

2.6. Northern and Western analyses

DBY747 wild-type cells were transformed with YEp-hMRE11B and transformants were selected on SD-Ura plates. Five independent colonies were cultured in 5–10 ml SD-Ura liquid media overnight until the cultures reached a titer of $2–5 \times 10^7$ cells/ml, at which time cells were collected by centrifugation. Total RNA was isolated by a rapid method as described (Schmitt et al., 1990), separated by electrophoresis and bloted to GeneScreen Plus nylon membrane (Du Pont). For multiple tissue Northern (MTN) analysis, MTN membranes were purchased from Clontech and used as instructed. The blots were hybridized with the [α-^{32}P]dCTP-labeled 0.5 kb fragment from the 5′-terminus of the *hMRE11B* cDNA. The *ACT1* gene was isolated as a 1.6 kb *Bam*HI–*Hin*dIII fragment from

pAA93 (a gift from Dr F. Sherman, Rochester University) and used as an internal control for yeast RNA.

The *mre11Δ* strain transformed with either pGBT-hMRE11B, pGBTMRE-Ch1 or pGBTMRE-Ch2, was grown in 10 ml SD-Trp liquid media overnight until the cell density reached $A_{600}=0.7$. Cells were harvested, washed with 5 ml of cold extraction buffer [50 mM Tris–HCl (pH 7.5); 30 mM KCl; 10% glycerol] and resuspended in 200 μl of the same solution containing the following concentrations of protease inhibitors: 1 μg/ml each of leupeptin, chymostatin, pepstatin A; 1 mM benzamidine and 1 mM phenylmethylsulfonyl fluoride. Glass beads were added and cells were disrupted by vortexing at top speed for a total of 5 min with 10 intervals on ice. Cell debris and glass beads were removed by centrifugation at 13 000 rpm for 5 min in an Eppendorf centrifuge at 4 C. The supernatant was collected and stored at −70°C until further use. The concentration of the crude cell extract was determined by the Bradford method using a protein assay kit from Bio-Rad Laboratories. Anti-Gal4 antibodies (Santa Cruz) were used at a dilution of 1:500 in 10 mM Tris–HCl (pH 7.5)–150 mM NaCl–2% BSA; 20 ml of this mixture was used to probe the nitrocellulose blots (8 × 10 cm) for 2 h at room temperature. The secondary antibody was affinity purified goat anti-rabbit (H + L) immunoglobulin G (Jackson ImmunoResearch Affinipure Antibodies obtained through Bio Can Scientific) conjugated to alkaline phosphatase, which was used at a 1:1500 dilution. The presence of an alkaline phosphatase-coupled immune complex was detected by a color detection method using a mixture of NBT (nitro blue tetrazolium chloride) and BCIP (5-bromo-4-chloro-3-indoloyl phosphate) as substrates (obtained from Bio-Rad Laboratories).

3. Results

3.1. Molecular cloning of hMRE11B cDNA

The yeast *MRE11* sequence was used to search the Human Genome Sciences Inc. database, and a putative clone (HGS360.301) from human corpus colosum cDNA showed significant homology with *yMRE11*. However, this clone, containing a 1.9 kb cDNA insert, appeared to be incomplete at the 5′ coding region. A HeLa cell cDNA library made in pSPORT1 was then screened to search for the full-length *hMRE11* using the 0.5 kb 5′ end of the HGS360.301 insert as a probe. A total of 16 putative *hMRE11* clones (HeLa 1–16) were obtained, among which the amino-acid sequence deduced from HeLa16 extends homology to the N-terminal yMre11. While our work was in progress, a *hMRE11* cDNA sequence was reported (Petrini et al., 1995). Therefore, HeLa16 cDNA was renamed *hMRE11B*. Although the 5′ non-coding regions of *hMRE11* and *hMRE11B* are highly diverse, *hMRE11B* sequence (GenBank accession number AF022778) within the coding region is almost identical to the reported *hMRE11*, with three exceptions: (i) it encodes a Val_{31} instead of Ala_{31}, which does not fall in a conserved region; (ii) it contains an 84 bp additional sequence resulting in an in-frame insertion of 28 amino acids Asp_{596}–Ala_{623}; and (iii) it contains a frameshift compared to *hMRE11* that results in an early translational termination of *hMRE11B* missing 28 amino acids at the C-terminus. These features of hMre11B result in a greater degree of homology with *Mus musculus* and *S. cerevisiae* Mre11 than the previously identified hMre11 (Fig. 1). Both HGS360.301 and HeLa16 contain the 84 bp insertion. We further analyzed some other partial HeLa *hMRE11* cDNA clones by restriction digestion taking advantage of the *Xba*I site within the 84 bp insert. All clones examined contain the *Xba*I site at the expected insertion region, indicating that this 84 bp insertion is not due to a clonal artifact. Recently, Paull and Gellert (1998) independently identified a new *hMRE11* from a human testis cDNA library. Their isolated clone showed the same $Ala_{31}Val$ amino-acid variation, the 84 bp insertion sequence and the frameshift resulting in an early termination like our *hMRE11B* cDNA, which confirms that these differences are indeed characteristic of *hMRE11* cDNA present human cells.

3.2. Expression of hMRE11B in human tissues and cancer cells

Examination of *hMRE11B* expression in different human tissues and cell cultures by Northern hybridization revealed the presence of two transcripts of approx. 2.6 and 7.5 kb (Fig. 2), with the 2.6 kb transcript consistent in size with the HeLa16 clone. In addition, a transcript smaller than 2.6 kb was observed only in skeletal muscles (Fig. 2A, Lane 6). Two transcripts were also observed in mouse cells (Petrini et al., 1995), a phenomenon common in most other meiotically expressed human genes (Dolganov et al., 1996; Kanaar et al., 1996) which appears to be the result of alternative splicing. Both the 2.6 and 7.5 kb transcripts were detected in different human tissues analyzed, with most normal tissues exhibiting extremely low levels of *hMRE11B*, some moderate expression in tissues such as lymph node, adrenal gland (Fig. 2B), and a relatively high-level expression in testis (data not shown). In contrast, the *hMRE11B* expression appears to be elevated in most cancer cell lines, including HeLa S3, lymphoma, colon carcinoma and all three leukemia cell lines examined (Fig. 2C). It is interesting to note that the expression of *hMRE11B* in bone marrow is signi cantly lower compared to leukemia cell lines (Fig. 2B, Lane 7 and 2C, Lanes 1, 3 and 4). However, one must

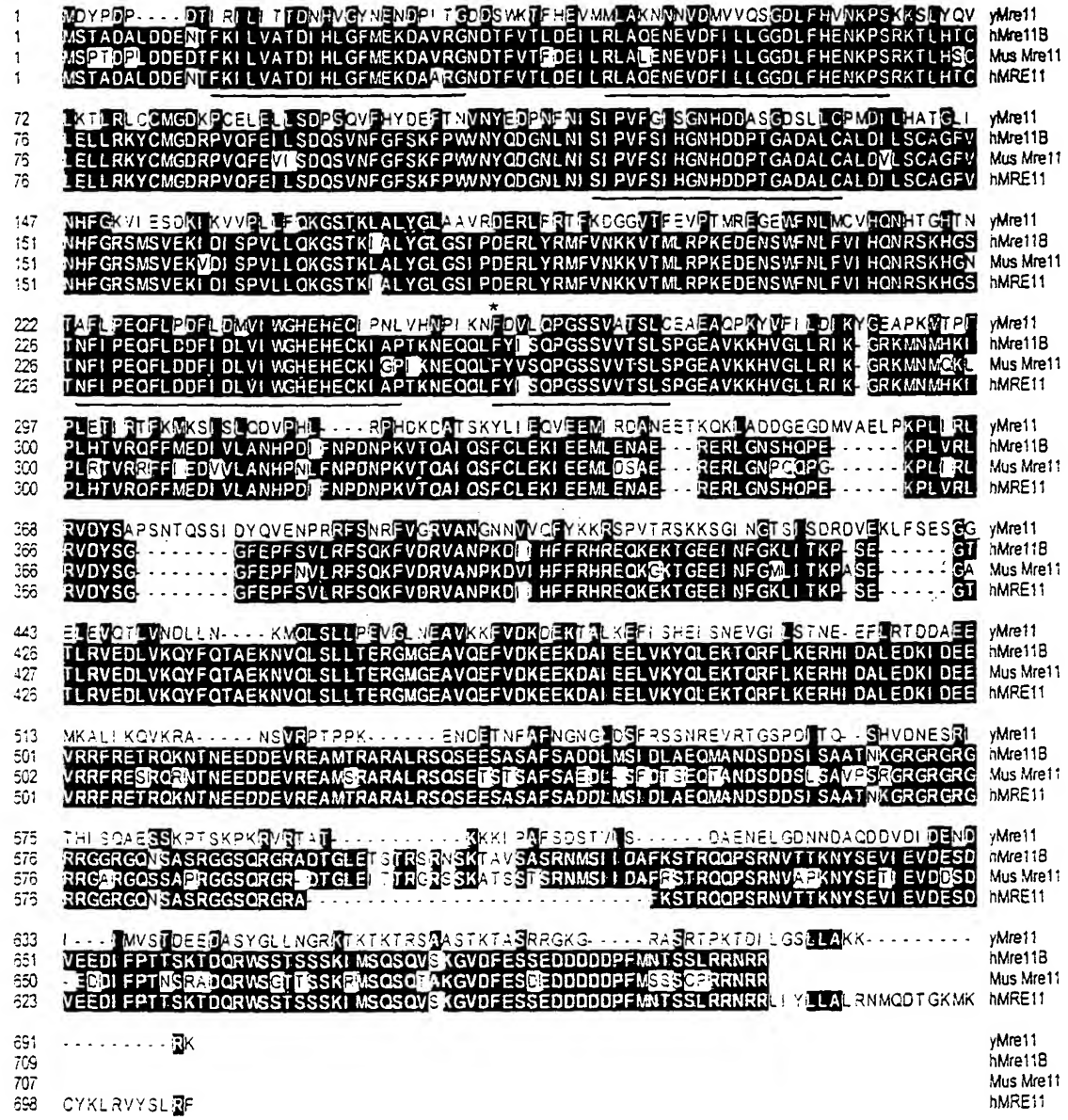

Fig. 1. Amino-acid sequence alignment of hMre11 (708 aa, U37359), *Mus musculus* (Mus) Mre11 (706 aa, U38987), hMre11B (708 aa, AF022778) and yeast (Sc)Mre11 (693 aa, D11463) using the Clustal method with PAM250 residue weight table. Identical amino-acid residues shared by two or more proteins are highlighted. The four putative phosphoesterase domains are underlined. The point at which the *hMRE11* ORF is fused to that of the *yMRE11* in *MRE-Ch1* and *MRE-Ch2* clones is shown by a star.

be cautious when comparing gene expression from cell cultures to that from normal tissues, because these cells are subject to different growth conditions and because cell cultures are derived from homogeneous cell types, whereas tissues may contain multiple cell types.

3.3. *Heterologous expression of* hMRE11B *in yeast cells*

In *S. cerevisiae*, mutations in *MRE11* cause pleiotropic phenotypes during vegetative growth, including an increased sensitivity to simple DNA alkylating agents and ionizing radiations, and hyper-recombination in certain mitotic recombination assays (Johzuka and Ogawa, 1995; Chamankhah and Xiao, 1998). During meiosis, the spore viability is reduced in *mre11Δ* homozygous diploids (Johzuka and Ogawa, 1995). In order to examine whether the homology between the primary structures of Mre11 and hMre11 extends to their functions, we attempted to rescue the MMS sensitivity of *S. cerevisiae mre11* mutants by expression of the *hMRE11B* cDNA. A multicopy plasmid carrying the *hMRE11B* cDNA driven by the *ADH1* promoter was unable to

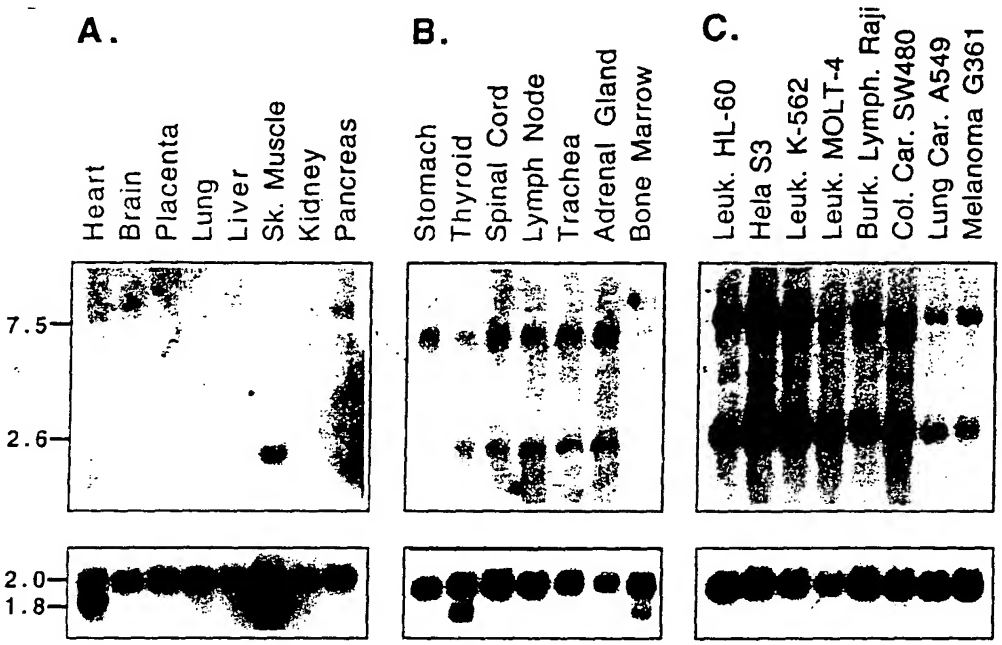

Fig. 2. Tissue-specific expression of *hMRE11B*. Membranes containing multiple tissue poly(A) RNA (2 μg per lane) were purchased from Clontech and used as instructed. (A) Human multiple tissue Northern (MTN) blot; (B) Human III MTN blot; and (C) Human Cancer Line MTN blot. Tissues and cell lines from which RNA was isolated are given on top of the figure. A 0.5 kb fragment from the 5′ end of the HGS360.301 insert was used as the *hMRE11B* probe (top panels). The 2.0 kb β-actin gene probe was supplied by Clontech as an internal control (bottom panels). Membranes A, B and C were hybridized and exposed to X-ray film under identical conditions.

rescue *mre11-ts* and *mre11Δ* cells from killing by MMS (Table 1). Similarly, Petrini et al. (1995) reported that *hMRE11* was unable to complement the γ-ray sensitivity of a *mre11* mutant strain. We further explored the reasons for this lack of complementation. First, we examined the expression of *hMRE11B* in yeast cells and found that the failure to complement the yeast *mre11* defect was not due to its lack of expression, since Northern and Western analyses both detected a high level of *hMRE11B* mRNA and protein, respectively (data not shown). Secondly, we tested the ability of hMre11B to self-interact. In a yeast two-hybrid assay, $Gal4_{DB}$-hMre11B and $GAL4_{AD}$-hMre11B fusion proteins were able to interact with each other (Fig. 3), consistent with the observed dimerization of yMre11

Table 1
hMRE11B does not complement yeast *mre11* mutants[a]

Transformant	% Survival on 0.005% MMS	% Survival on 0.015% MMS
mre11Δ/YEp127	25	5
mre11Δ/YEp-hMRE11B	27	4
mre11-ts/YEp127[b]	100	48
mre11-ts/YEp-hMRE11B[b]	100	45

[a] Results are the average of two independent experiments with standard deviations < 10%.
[b] *mre11-ts* transformants were incubated at 34 C non-permissive temperature.

(Johzuka and Ogawa, 1995), albeit at a substantially lower level. We also tested the possibility that hMre11B forms a counter-productive complex with yeast proteins, and hence confer a dominant-negative effect. This type of effect has been reported previously on some mammalian Rad51 and Rad52 homologs (Milne and Weaver, 1993; Donovan et al., 1994). Fig. 4 shows the result of a killing experiment with wild-type cells overexpressing *hMRE11B*. Clearly, no dominant-negative effect of *hMRE11B* in the wild-type yeast cells was observed, indicating that hMre11B, although proficient in dimerization does not efficiently compete for the yeast Mre11–Rad50–Xrs2 complex formation. Consistent with this result, we found that neither of the hMre11B fusion constructs was able to interact with yMre11, yRad50 and yXrs2 proteins in the same assay (Table 2). It should be noted that *hRAD51* and *Kluyveromyces lactis RAD51* are unable to complement the corresponding *S. cerevisiae rad51* null mutants (Donovan et al., 1994; Shinohara et al., 1993), and that the hRad52 domain required for interaction with hRad51 is not conserved in yRad52 (Shen et al., 1996). Thus, the function of eukaryotic DNA recombinational repair multiprotein complexes appears to be highly species-specific.

3.4. Domain(s) required for hMre11B dimerization

To further address if the species specificity of Mre11 is due to lack of conservation within the protein inter-

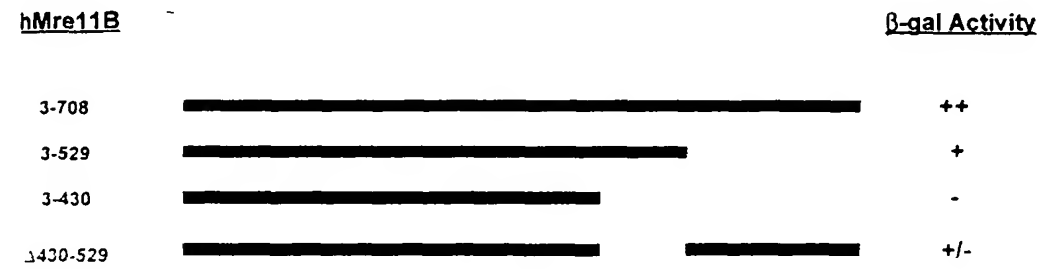

Fig. 3. The hMre11B dimerization by a two-hybrid assay. The *hMRE11B* open reading frame was cloned into both pGBT9 and pGAD424 and co-transformed into *S. cerevisiae* haploid Y190 as a positive control. Plasmid pGBT-hMre11B was used to make hMre11B C-terminal deletions and the resulting plasmids were co-transformed with pGAD-hMRE11B. The transformants were plated onto SD-Trp-Leu, incubated at 30°C, and the β-galactosidase activity was determined by a filter assay as described (Bartel and Fields, 1995). Color development is indicated as follows: ++, 4–8 h; +, 8–16 h; +/−, 16–24 h; and − if no color developed after a 24 h incubation. Bars represent expected hMre11 polypeptides after deletion.

action domain(s), we attempted to map the hMre11B self-interaction domain. The preliminary results shown in Fig. 3 indicate that amino-acid residues 430–530 are required for hMre11B dimerization; in addition, the C-terminal 180 amino acids, where 28 amino acids are absent in hMre11 (Fig. 1), are also required to maintain the full activity. This result agrees with extensive deletion analysis of yMre11 (data not shown), suggesting that Mre11 dimerization requires multiple domains of the protein.

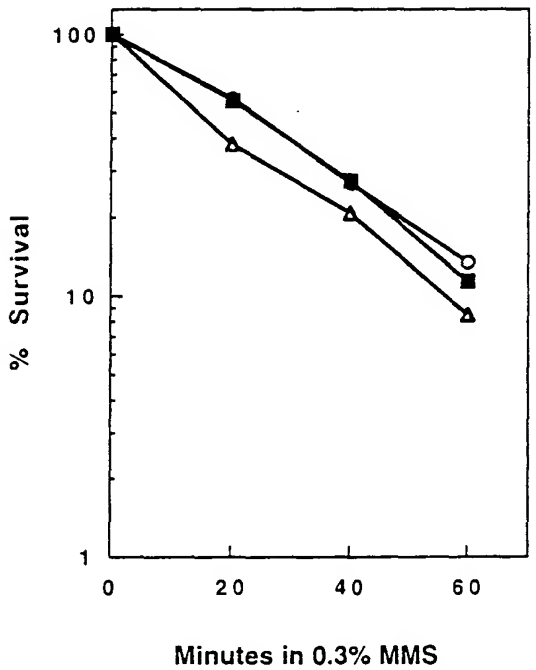

Fig. 4. Overexpression of *hMRE11B* in wild-type cells. DBY747 wild-type cells transformed with YEp-hMRE11B were grown overnight in selective media and treated with MMS. At 20 min intervals, samples were washed with distilled water and after serial dilution plated on YPD plates. Colonies were counted after 3–5 days of incubation at 30°C. The results are the average of two independent experiments with standard deviations < 10%. The plasmid retention rate of the YEp-hMRE11B transformants was determined by plating cells onto SD + Ura, and found to be 91 ± 4%.

3.5. Chimeric Mre11

Since the C-terminus of hMre11 and yMre11 are less conserved (Fig. 1), an obvious explanation for the lack of complementation may be the absence of sequence homology at the C-terminus between these two proteins. In order to test if the C-termini of Mre11 contain species-specific signals for complex formation, a chimeric *MRE11* clone (*MRE-Ch1*) was constructed in which the 5′ half of *hMRE11* was fused to the 3′ half of *yMRE11*. In this chimeric *MRE11* clone, all four phosphoesterase motifs thought to be important for Mre11 function (Sharples and Leach, 1995) are present, and the only differences with the yeast counterpart lie in the highly conserved N-terminal domain. The *MRE11-Ch1* clone

Table 2
Two-hybrid interactions of the yeast and human *MRE11* constructs

BD fusion construct	AD fusion construct	β-gal Activity[a]
pGBT9	pGAD424 or pACTII	−
pGBT9	pGAD-hMRE11B	−
pGBT-hMRE11B	pGAD424	−
pGBT-hMRE11B	pGAD-hMRE11B	+ +
pGBT-hMRE11B	pGAD-yMRE11	−
pGBT-yMRE11	pGAD-hMRE11B	−
pGBT-yMRE11	pGAD-yRAD50	+ + +
pGBT-hMRE11B	pGAD-yRAD50	−
pLexA-yMRE11	pGAD-yXRS2	+/−
pGBT-hMRE11B	pGAD-yXRS2	−
pGBTMRE-Ch1	pGAD-hMRE11B	−
pGBTMRE-Ch1	pGAD-yMRE11	−
pGBTMRE-Ch1	pGAD-yRAD50	−
pGBTMRE-Ch2	pGAD-hMRE11B	−
pGBTMRE-Ch2	pGAD-yMRE11	−
pGBTMRE-Ch2	pGAD-yRAD50	−

[a] β-Galactosidase (β-gal) activity is as defined in Materials and Methods. The results were obtained with at least five independent transformants.

was unable to complement the MMS sensitivity of the *mre11Δ* strain. In addition, Mre11-Ch1 was unable to interact with either yMre11, yRad50 or hMre11B (Table 2). These results suggest that both the N-terminus and the C-terminus of Mre11 are required for species-specific protein–protein interactions. As a control, we constructed a second chimeric *MRE11* in which the N-terminus of *y.MRE11* was fused to the C-terminus of *h.MRE11*. We showed that this clone is also unable to complement the MMS sensitivity of *mre11Δ*, nor could it interact with the yMre11, yRad50 or hMre11B (Table 2). This result again confirms our conclusion that the functional and interacting domains of Mre11 protein both act in a species-specific manner.

4. Discussion

We cloned the *h.MRE11B* cDNA as a homolog of yeast *MRE11*. Our *h.MRE11B* differs from the published *h.MRE11* cDNA (Petrini et al., 1995). The major difference lies in the 84 bp insertion in *h.MRE11B*, encoding additional 28 amino acids compared to hMre11. In addition, the 5′ untranslated region of *h.MRE11B* cDNA does not share sequence homology with that of *h.MRE11*. It has not been determined whether these two cDNAs are the result of alternative splicing of the same gene, represent the *h.MRE11* gene from different tissues/sources, or if they are encoded by two duplicated genes. It is interesting to note that fluorescence in situ hybridization identified two signals in chromosomes 7 and 11 (Petrini et al., 1995), suggesting that normal human cells may indeed contain two copies of the *hMRE11* gene.

To the best of our knowledge, the tissue-specific expression of human *MRE11* gene has not been explored. The expression pattern of *h.MRE11* in different tissues revealed that, unlike mouse *MRE11* (Petrini et al., 1995), the *hMRE11* transcript level in most of the tissues examined is relatively low. In contrast, a number of cancer cell lines express high levels of *hMRE11* compared to normal tissues. This difference may imply certain role(s) for *hMRE11* in tumorogenesis. Interestingly, Mre11, along with Rad50 and Xrs2, is involved in DNA non-homologous (illegitimate) end joining of double-strand breaks (DSB) (Tsukamoto et al., 1996, 1997), acting as an alternative pathway for the repair of DSBs in yeast. It is conceivable that an increase in illegitimate end-joining activity may facilitate chromosome translocation. Indeed, Honma et al. (1997) demonstrated that illegitimate recombination contributes to the generation of allelic loss and genomic instability in p53-mutated cells. Another study (Dar et al., 1997) suggests that there is a defective illegitimate recombination pathway in ataxia-telangiectasia cells and indicates that the characteristic radiosensitivity and genomic instability phenotypes of such cells could be attributed to a defect in an illegitimate recombination dealing with DSBs. In addition, yeast Mre11 and ot components in the non-homologous DNA end-joining pathway are also shown to function in telomere maintenance (Boulton and Jackson, 1998; Nugent et al., 1998). Cancer cells exhibit characteristic telomere dynamics and their chromosomes usually have shorter telomeres and a higher telomerase activity than normal cells (Ishikawa, 1997). Hence, altered expression of genes involved in telomere maintenance can cause telomere shortening, a process that eventually destabilizes the ends of chromosomes, leading to genomic instability (Counter, 1996; Gravel et al., 1998).

We wished to study the structure–function relationship of hMre11 in yeast cells. However, like *h.MRE11* (Petrini et al., 1995), *hMRE11B* can not functionally substitute for the yeast *MRE11*. We then addressed possible reasons for this lack of complementation. Our results from the yeast two-hybrid analyses and dominant-negative studies support the notion that hMre11, although capable of self-interaction, is incapable of forming a functional complex with cognate yeast proteins Mre11, Rad50 and Xrs2. Assuming that the dimerization of Mre11 protein is a prerequisite for assembly into a functional multi-protein complex, it seems that the dimerization alone is insufficient for hMre11B to interact with other yeast proteins, thus unable to form a functional complex. Instead, the function of th protein complex appears to be species-specific. Indeed, recent cloning and characterization of the human *NBS1* gene indicates that defects in its protein product, Nibrin, is involved in Nijmegan breakage syndrome (Varon et al., 1998) and that Nbs1 appears to be a functional counterpart of yeast Xrs2. However, the two proteins do not possess significant sequence homology (Carney et al., 1998). This result is in good agreement with our observations by yeast two-hybrid analyses that hMre11B does not interact with yXrs2.

The inability of both chimeric *MRE11* constructs to functionally complement the MMS sensitivity of yeast *mre11* mutants demonstrates that the lack of conservation at the C-terminus of Mre11 alone cannot account for the lack of complementation. In support of this notion, we showed that both chimeric *MRE11* are also deficient in dimerization as well as interaction with Rad50. These observations, along with previous two-hybrid analyses of human and yeast Mre11, allow us to draw three important conclusions. First, although the N-terminus of Mre11 is an important domain in terms of function and interaction, the conserved phosphoesterase motifs alone are insufficient for protein–protein interaction and in vivo functions. Secondly, Mre11 self-interaction and function are species-specific. Lastly, the inability of chimeric Mre11 to form a dimer indicate either that Mre11 dimerization is via N-to-C-terminal

contact, or that it requires proper folding of each Mre11 monomer, which in turn involves both the N- and C-termini. The fact that chimeric Mre11 clones are unable to interact with yMre11 or hMre11B is consistent with the second hypothesis.

The species-specificity of Mre11 and lack of sequence conservation between Nbs1 and Xrs2 may also reflect different functions of this complex in human and yeast cells. This possibility is strengthened in light of the fact that in mammalian cells, illegitimate recombination is the preferred recombination pathway for dealing with DSBs, as opposed to *S. cerevisiae*, where homologous recombination acts as the predominant end-joining pathway (Roth and Wilson, 1985; Godwin et al., 1994).

Based on the results presented in this study, we conclude that biological systems may employ different strategies to function in a species-specific manner, and that the absence of primary sequence conservation between two species may not be indicative of a lack of functional conservation. Despite the fact that DNA repair proteins from different species may not display a significant primary structure homology, these proteins are indeed functionally conserved between lower and higher eukaryotes, as is evident from biochemical analyses. In the case of hRad52 and hRad51 interaction, the primary structure of hRad52 interacting domain is responsible for species-specificity, whereas in the hMre11/hRad50 complex, the absence of secondary structure conservation governs the species specificity between yeast and human. Future biochemical experiments on the purified proteins will further address this issue.

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